SIZE AND SHAPE OF ISOLATED PROTEINS OF THE SMALL RIBOSOMAL SUBUNIT OF RAT LIVER

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Received 17 August 1979

1. Introduction

Determinations of the size and shape of individual ribosomal proteins in solution are a prerequisite to the understanding of their spatial arrangement and relationship in ribosomal particles. Such studies by means of hydrodynamic and scattering methods, using in the latter case X-rays and neutrons, have been performed to now with proteins of prokaryotic ribosomes only [1–8]. Comparable experiments and data about isolated single proteins of eukaryotic ribosomes are not yet available. First indications of more or less elongated shapes of eukaryotic ribosomal proteins in situ were obtained by immuno-electron microscopic studies [9–11].

Here results of experiments about the size and shape of individual proteins of the small subunit of rat liver ribosomes in solution, using hydrodynamic methods are described. It could be demonstrated that most ribosomal proteins are elongated with largest diameters of 7.7–19.4 nm.

2. Materials and methods

Single pure ribosomal proteins were prepared as in [12,13]; their purity, checked by two-dimensional polyacrylamide gel electrophoresis [12,13], was >95%. For the enumeration of the proteins the nomenclature of [14] will be used. For the hydrodynamic investigations the proteins were dissolved in concentrations of 0.3–1.0 mg/ml 10 mM β-mercaptoethanol in absence or in presence of 0.1 M KCl. Sedimentation and diffusion measurements were performed in a Beckman Model E ultracentrifuge with ultraviolet-absorption optics, monochromator and photoelectric scanner using a capillary-type double-sector synthetic boundary cell. From the moving boundary at 40 000 or 48 000 rev./min, recorded at λ = 280 nm, sedimentation coefficients were calculated. Diffusion coefficients were determined with the same technique at rotor speeds of 12 000 rev./min (AnH-rotor) by measuring the broadening of the boundary in relation to the speed and dilution in the cell. The partial specific volumes of the proteins were determined from the increments of amino acids [15] and the amino acid composition of the proteins [16]. Molecular weights (M) were calculated using the Svedberg formula and the frictional ratio (f/f_o) from sedimentation (s) and diffusion (D) coefficients and the partial specific volumes ($\bar{v}$) by the equation:

$$f/f_o = 10^{-8} \left(\frac{1-\rho\bar{v}}{D^2.s.\bar{v}} \right) 1/3$$

where ρ = density of solvent

Assuming prolate ellipsoids and an average amount of 0.3 g bound water/g protein from the frictional ratio a ratio of the half-axes $a:b$ can be estimated from the diagram in [17]. Considering these values and the volumes (V) of each protein:

$$V = \frac{M.\bar{v}}{N_L}$$

where N_L = Avogadro's number

the greatest length or diameter ($2a$) as well as the mass/length ratio ($M/2a$) were calculated, too.

3. Results and discussion

Sedimentation coefficients of the isolated ribosomal proteins are relatively small because of their more or less extended shape. The values vary fractionally in solutions of low concentration. By extrapolation to concentration $c = 0$, sedimentation constants ($s^0_{20,w}$) can be obtained (see table 1). No influence of the protein concentration on the D values could be found. Therefore these are average values of different experiments ($\bar{D}_{20,w}$). The molecular weights of proteins investigated are in the range of $35-10 \times 10^3$ (see table 1). Similar results were obtained by two-dimensional polyacrylamide gel electrophoresis [18]. The frictional ratios and consequently the estimated ratios of half-axes ($a:b$), the greatest diameters ($2a$) and the the mass/length ratios ($M/2a$) vary considerably between the proteins studied (see table 1).

Considering these parameters the following conclusions can be drawn. Proteins S2, S28, S6, S13, S9, S3 and S8 are relatively extended proteins with a nearly worm-like shape. The ratio of their half-axes varies between 9.5:1 and 5.5:1, respectively. On the other hand, proteins S17, S19, S21 and S25 are less extended; the ratio of their half-axes vary between 3:1 and 4.5:1. Thus, the latter proteins seem to be of more globular shape. In fact many proteins are not organized as ellipsoids of revolution but have the shape of three-axised bodies [19] so that the largest diameters ($2\alpha_{max}$) could be assumed to be somewhat shorter than those given in table 1. On the other hand, proteins S3 and S17 seem to be more compact (higher mass/length ratio) than S2, S13, S21 or S28 (see last column of table 1).

These results are in good agreement with our findings about the shape and size of proteins in the small ribosomal subunit derived from data obtained by immuno-electron microscopy [11,20]. The greatest distance between two of the three observed antigenic determinants of protein S2 was found to be ~12 nm and that of protein S3 ~8 nm. In solution the greatest diameters of these proteins were estimated by hydrodynamic methods to be 19.4 nm and 13.6 nm, respectively. Similar data could be obtained also for proteins S6, S7 (unpublished results), S17 and S21 [11].

Proteins S17 and S21 [11] show two neighbouring antigenic determinants on the surface of the small ribosomal subunit. The diameter of one antigen combining site of an antibody molecule (IgG) is ~4 nm [21,22]; the length of the proteins S17 and S21 was estimated by hydrodynamic methods to be 7.7–8.0 nm. Comparing these values, proteins S17 and S21 localized on the surface of the small ribosomal subunit should be covered nearly completely by two antigen combining sites of two different antibody molecules. In fact two antibody molecules against proteins S17 and S21 each were bound to the 40 S ribosomal subunit as demonstrated by immuno-electron microscopy [11]. The good agreement of the hydrodynamic results with the findings from

Table 1
Hydrodynamic parameters of individual ribosomal proteins of rat liver

Protein [14]	$s^0_{20,w}$ (S)	$\bar{D}_{20,w}$ (F)	$\bar{\nu}$ (ml/g)	$M_{s,D}$	f/f_o	$a:b$	$2a_{max}$ (nm)	$M/2a_{max}$ (nm^{-1})
S2	2.12	5.88	0.7390	33 700	1.69	9.5:1	19.38	1739
S3	2.52	6.81	0.7421	34 950	1.44	5.5:1	13.55	2579
S6	2.20	6.47	0.7394	31 800	1.56	7 :1	15.40	2065
S7	2.12	7.64	0.7433	27 300	1.41	5 :1	11.72	2329
S8	2.19	7.40	0.7321	26 900	1.45	5.5:1	12.36	2176
S9	1.98	7.37	0.7379	25 000	1.49	6 :1	12.82	1950
S13	1.78	7.33	(0.734)[a]	22 000	1.55	7 :1	13.64	1628
S17	2.20	9.26	0.7330	21 700	1.25	3 :1	7.70	2818
S19	1.99	9.40	0.7308	19 150	1.28	3.8:1	8.62	2222
S21	1.69	10.06	0.7132	14 200	1.33	4 :1	8.00	1775
S25	2.04	8.36	0.7396	22 800	1.35	4.5:1	10.28	2218
S28	1.05	8.74	0.7271	10 700	1.65	8.5:1	12.12	883

[a] Calculated by extrapolation

immuno-electron microscopy, so far as has been investigated, indicate that no major differences seem to exist in the shape of the ribosomal proteins in solution as well as when assembled in the ribosomal particle. Nevertheless, differences in the secondary structure, as observed in the case of ribosomal proteins of *Escherichia coli* [23] can not be excluded at present.

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